Saving Species With Science®

CREW PROGRESS REPORT

Lindner Center for Conservation and Research of Endangered Wildlife

Cincinnati Zoo & Botanical Garden • Spring 2023

Scientific Breakthrough at CREW Gives Community Cats a Shot

Scan the QR code for more information.

A groundbreaking study conducted by CREW scientists has validated a nonsurgical sterilization method for domestic cats, representing a major milestone toward humanely reducing free-roaming cat populations and eliminating shelter euthanasia of healthy cats. The research results were recently published in Nature Communications, a prestigious peer-reviewed, open-access, multidisciplinary science journal (*scan the QR code to access the full paper*). There are currently ~80 million community (i.e., unowned outdoor) cats in the United States. Many of these cats experience stressful lives and face possible euthanasia in animal shelters. Animal welfare experts have long identified sterilization as a key component to humanely reducing community cat populations. Currently, surgical spay and neuter procedures are the standard for sterilizing cats. However, surgery requires general anesthesia, an adequately equipped surgical facility, and more veterinarians than are currently available. A nonsurgical sterilant would address the limitations of surgical sterilization and empower animal welfare organizations to solve this monumental problem. A promising new approach involves a hormone termed AMH (Anti-Müllerian Hormone) that is naturally produced by the ovaries to regulate the number of eggs that develop during each reproductive cycle. When present at high levels, AMH is a potent inhibitor of egg development and represents an attractive candidate for inducing non-surgical sterilization. In collaboration with Massachusetts General Hospital, CREW investigated a novel approach for producing AMH: recombinant adeno-associated virus (AAV)–delivered gene therapy. Our collaborators developed an AAV construct that encodes for the AMH protein, essentially borrowing the protein-making machinery of the cat's muscle cells to produce the hormone. In our study, six female cats at CREW were treated with the gene therapy at two different doses, and three cats served as controls. Excitingly, we found that a single injection of the gene therapy treatment causes the cats' muscles to produce AMH (which is normally only produced in the ovaries) and raises the overall level of AMH about 100 times higher than normal. Two 4-month-long breeding trials were performed one and two years post-treatment to test the efficacy of the AMH gene therapy. All the control cats produced kittens, but none of the cats treated with the gene therapy became pregnant. Furthermore, the cats' AMH levels have remained elevated for 3+ years since initial treatment, indicating that our gene therapy treatment may be able to provide contraception for the rest of the cats' lives. These findings suggest that in a few years, it may be possible to sterilize female cats with a simple injection, allowing for broader application to owned and unowned cat populations without the need for anesthesia or surgery.

(Funded by the Joanie Bernard Foundation and Michelson Found Animals Foundation)

"Using Science to Learn, Applying Knowledge to Save, A Future for Wildlife"

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First AI Rhino Calf to Successfully Reproduce—a Product of CREW's Rhinoceros *Signature* Project

CREW started working on developing artificial insemination (AI) procedures for greater one-horned (GOH) rhinos in 1998. A decade later, our first AI calf was produced at the Cincinnati Zoo but sadly did not survive. Subsequent, successful AI procedures conducted at Cincinnati and other zoos resulted in a total of seven GOH calves. Meanwhile, Europeans and the San Diego Zoo Wildlife Alliance produced ten white rhinos by AI. Of those 17 rhino AI calves worldwide, 13 are still alive, and 7 are currently of reproductive age, but none had reproduced. That changed in February 2023 when female GOH rhino Monica gave birth to a healthy calf after breeding naturally with her mate 14 months earlier at the Tanganyika Wildlife Park in Kansas. Rhino "Monica" was named for the CREW scientist who performed the AI procedure that resulted in her birth at the Buffalo Zoo in 2014, but what's most exciting about this recent birth is the fact that Monica was a product of rhino sperm stored in CREW's CryoBioBank for 10 years after the donor male rhino had died. That male rhino "Jimmy" was a Cincinnati Zoo resident but never sired offspring during his lifetime. Because of CREW's reproductive expertise and the dedicated Buffalo Zoo and Tanganyika staff, Jimmy's genes now live on both in rhino mom Monica and her male calf Marjon (a.k.a., "Jimmy Jr" to us).

Tackling the Secrets of Cycads—the World's Most Threatened Group of Plants

Threatened by illegal collecting, habitat loss, disease, and climate change, the more than 300 species of cycads are considered the most highly threatened group of plants on earth. They are also exceptional species and can't be conserved by seed banking. With support from BGCI's Global Botanic Garden Fund, CREW's Plant Division is developing alternative methods for cryobanking cycad tissues using a method demonstrated in the 1990s by Professor Richard Litz from Florida. He showed that cycads can produce somatic embryos from young leaf tissue and these embryos can be cryopreserved and germinated into plants. CREW tested leaf tissues of 82 samples, received from the Montgomery Botanical Center, on his somatic embryogenesis medium. Over half (55%) of those samples that remained clean in culture produced significant growth. Much of the growth appeared to contain "pro-embryos" or very young, nascent somatic embryos, similar to those reported in the 1990s. These represented 13 species of *Ceratozamia*, *Cycas*, and *Zamia*. The development of full embryos may take more months of culture, but this work has already shown leaf tissues of many cycad species are responsive to this medium and hold potential for producing tissues that could ultimately be used for conservation cryobanking.